

1061-Symp Monitoring Translation Kinetics One Ribosome at a Time Ignacio Tinoco.

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We have used optical tweezers to monitor the codon-by-codon translation of a messenger RNA by a single ribosome. A base-paired stem-loop (a hairpin) is held between two beads; the force on the mRNA and the distance between the beads is measured as a function of time. As translation proceeds through the hairpin, the end-to-end extension of the RNA increases in a trajectory of pause-translocation-pause... steps. The pauses are of order seconds; the translocation steps correspond to a movement of the ribosome by three nucleotides, and occur in less than 25 ms. The mean pause times, and their distribution depend on force on the messenger RNA; lower forces cause longer pauses. Force on the ends of the hairpin lower the barrier to translation. Decreasing the concentrations of elongation factors, EF-G and EF-Tu, increase the pause times, as expected. However, we have not yet found any effect of force, or of concentrations of elongation factors, on translocation times.

1062-Symp Structural and Functional Dynamics of Nucleic-acid Interacting Enzymes Studied by Single-molecule FRET Xiaowei Zhuang. HHMI, Harvard Univ, Cambridge, MA, USA. TBA

Platform Q: Interfacial Protein-Lipid Interactions

1063-Plat Energetics Of Peptide (pHLIP) Binding To And Folding Across A Lipid Bilayer Membrane

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The pHLIP peptide (pH Low Insertion Peptide) serves as a model system for peptide insertion and folding across a lipid bilayer. It has three general states: (I) soluble in water or (II) bound to the surface of a lipid bilayer as an unstructured monomer, and (III) inserted across the bilayer as a monomeric α -helix. We used fluorescence spectroscopy and isothermal titration calorimetry to study the interactions of pHLIP with a POPC lipid bilayer and to calculate the transition energies between states. We found that the Gibbs Free Energy of binding to a POPC surface at low pHLIP concentration (state I - state II transition) at 37°C is about -7 kcal/mol near neutral pH and that the free energy of insertion and folding across a lipid bilayer at low pH (state II - state III transition) is nearly -2 kcal/mol. We plan to discuss a number of related thermodynamic parameters from our measurements. Besides its fundamental interest as a model system for the study of membrane protein folding, pHLIP has utility as an agent to target diseased tissues and translocate molecules through the membrane into the cytoplasm of cells in environments with elevated levels of extracellular acidity, as in cancer and inflammation. The results give the amount of energy that might be used to move cargo molecules across a membrane.

1064-Plat A Machine Learning Protocol for Distinguish Intra-domain Peripheral Membrane Targeting Properties using Sequence and Structure Morten Kallberg, Hui Lu.

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Peripheral membrane-targeting proteins can associate with membranes in a reversible manner, allowing for the transient localization of these proteins to specific intracellular sites. Due to this property, such proteins are often found to be important players in both signal transduction and protein trafficking processes. A number of domain families, for example C1-, C2-, and PH-domains, have been found to be of great importance in driving this type of association, however, no specific sequence motifs eluding to the targeting properties have been identified in these families. For this reason, a simple procedure based on sequence similarity alone will not be effective in computational function annotation.

We present a machine learning protocol for distinguishing intra-family membrane-targeting properties. The protocol is based on features obtained from both sequence and structure allowing for the incorporation of both statistics obtained from the entire domain family as well as physical quantities specific

to each domain. First, values for residue conservation in targeting versus non-targeting domains are calculated. Second, properties such as electrostatics and surface hydrophobicity of the domain are quantified by defining patches of similar values on the solvent exposed surface of the structure. Based on these features we construct a classification model for each family and furthermore compare the performance of a number of algorithms in this problem domain.

Finally, we explore the interdependence of the features in determining the membrane-targeting properties of each family through the use of alternating decision trees, drawing out the specific targeting properties for each family.

1065-Plat Peripheral Protein Organization on Biomimetic Membranes: Protein-protein and Protein-lipid Interactions

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Protein organization on the plasma membrane and their dynamics are essential for cellular function; however it is not clear how protein binding arranges the underlying lipids or how the membrane structure leads to functional protein organization. To answer these fundamental questions, we are using fluorescence imaging and fluorescence correlation spectroscopy to investigate the effects of annexin A5 binding to biomimetic membranes. Annexin A5, a peripheral intracellular protein that plays an anti-coagulation role, binds specifically to anionic (e.g., phosphatidylserine) membranes in the presence of calcium, with an equilibrium dissociation constant of 8 nM, as assessed by quartz crystal microbalance measurements. We find that annexin A5 exhibits clustering in addition to a more dispersed population when it binds to biomembranes, which is reflected in lateral diffusion coefficients of $\sim 10^{-10}$ cm²/s and $\sim 10^{-8}$ cm²/s, respectively. These two populations are also observed for the lateral diffusion of the head-group-labeled Texas Red-phosphatidylethanolamine (TR-PE) when found under clustered annexin A5. We then investigated the effect of annexin binding on the lateral diffusion specific acyl chain-labeled phospholipid analogs, NBD-phosphatidylcholine (NBD-PC) and NBD-phosphatidylserine (NBD-PS). We find that the numbers of both NBD-labeled lipids are greater under annexin clusters, as compared to off-annexin clusters. However, only NBD-PS exhibits two-component lateral diffusion under annexin clusters, whereas NBD-PC is unaffected whether it is measured on or off of annexin clusters. We are also investigating these effects in the presence of cholesterol-containing anionic membranes and find that annexin binding induces phase separation. These results suggest that upon binding to membranes, the peripheral protein annexin organizes the underlying lipids into domains, which may have functional implications *in vivo*.

1066-Plat Plasmon Waveguide Resonance Shows Preferential Binding of Oligomeric α -Synuclein to Raft-Like Lipid Mixtures

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α -Synuclein is a presynaptic protein whose fibrillar and β -sheet rich aggregates are implicated in several neurodegenerative diseases such as Parkinson's disease (PD). Different lines of evidence suggest that oligomer intermediates rather than monomeric or mature fibrillar deposits constitute the toxic species, probably by membrane incorporation and pore formation [1]. We used plasmon waveguide resonance (PWR) spectroscopy [2] to characterize the binding of various α -Synuclein aggregates to planar lipid membranes. The binding isotherms yielded affinity constants for the membrane-active aggregation species of α -Synuclein. In addition, using different lipid mixtures, we studied the role of the lipid composition for membrane insertion of the α -Synuclein oligomers. To mimic the compositional and structural heterogeneity of neuronal membranes we used detergent-induced membrane fusion and employed raft-like mixtures of sphingomyelin, eggPC, and cholesterol. These mixtures exhibit stable lateral domain formation as revealed by solid-state ²H NMR [3]. The results show that binding and membrane insertion of α -Synuclein is highly dependent on the aggregation state of the protein. Our data suggest that the lateral segregation into lipid domains strongly promotes membrane insertion of the toxic aggregation species. Furthermore, we show that membrane lipids are able to dissolve pre-aggregated fibrils back into intermediate species. We therefore propose that the pathogenicity of α -Synuclein is highly dependent on the lipid composition of intracellular membranes, most notably the membranes of synaptic vesicles, and that the macroscopic aggregates found in PD patients act as a reservoir for toxic intermediates *in vivo*.

[1] Beyer, K. (2007) *Cell. Biochem. Biophys.* **47**, 285-299.

[2] Salamon, Z. *et al* (1999) *Trends Biochem. Sci.* **24**, 213-219.

[3] Bartels, T. *et al* (2008) *J. Am. Chem. Soc.*, in press.